

What is claimed is:

1. A method of generating antigen specific allospecific human suppressor CD8+CD28- T cells which comprises:

a) obtaining peripheral blood T cells from a subject;

b) stimulating by multiple priming a T cell line from the T cells obtained in step (a) with allogeneic antigen presenting cells (APCs), said APCs expressing an MHC class I antigen recognized by the primed T cell line and an MHC class II antigen recognized by CD4+ T helper cells from said primed T cell line;

c) isolating primed CD8+ T cells and CD4+ T helper cells from the T cell line stimulated in step (b);

d) isolating primed CD8+CD28- T cells from the isolated primed CD8+ T cells of step (c);

e) detecting suppression by the primed CD8+CD28- T cells isolated in step (d) of interaction between the CD4+ T helper cells isolated in step (c) and allogeneic antigen presenting cells (APCs) expressing the same MHC class I antigen and the same MHC class II antigen expressed by the APCs used to stimulate the T cell line of step (b), thereby identifying antigen specific allospecific human suppressor CD8+CD28- T cells; and

f) expanding in culture the antigen specific allospecific human suppressor CD8+CD28- T cells identified in step (e), thereby generating the antigen specific allospecific human suppressor CD8+CD28- T cells.

2. The method of claim 1 wherein the MHC class I antigen is an HLA-A or HLA-B antigen expressed by the APC used for priming in step (b).

3. The method of claim 1 wherein the MHC class II antigen is an HLA-DR, HLA-DQ or HLA-DP antigen.

4. Antigen specific allospecific human suppressor CD8+CD28+ T cells produced by the method of claim 1.

5. A method of generating xenospecific human suppressor CD8+CD28- T cells which comprises:

a) obtaining peripheral blood T cells from a human subject;

b) stimulating by multiple priming a human T cell line from the T cells obtained in step (a) with a xenogeneic antigen presenting cells (APCs), said APCs expressing a xenogeneic MHC class I antigen and a xenogeneic MHC class II antigen;

c) isolating primed human CD8+ T cells and human\_CD4+ T helper cells from the T cell line stimulated in step (b);

5 d) isolating primed human CD8+CD28- T cells from the isolated primed human CD8+ T cells of step (c);

10 e) detecting suppression by the primed human CD8+CD28- T cells isolated in step (d) of interaction between the human CD4+ T helper cells isolated in step (c) and xenogeneic antigen presenting cells (APCs) expressing the same xenogeneic MHC class I antigen and xenogeneic MHC class II antigen expressed by the xenogeneic APCs used to stimulate the human T cell line of step (b), thereby identifying xenospecific human suppressor CD8+CD28- T cells; and

15 f) expanding the xenospecific human suppressor CD8+CD28- T cells identified in step (e), thereby generating the xenospecific human suppressor CD8+CD28- T cells.  
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25 6. The method of claim 5 wherein the xenospecific mammalian antigen presenting cells (APCs) are selected from pig or primate APCs.

30 7. The method of claim 5 wherein the xenogeneic MHC class I antigen is selected from the group consisting of swine histocompatibility leukocyte antigen (SLA) class-I and MHC class II antigen is selected from the group consisting of swine histocompatibility leukocyte antigen (SLA) class-II.

8. Xenospecific human suppressor CD8+ CD28+ T cells produced by the method of claim 5.

5 9. A method of generating allopeptide antigen specific human suppressor CD8+CD28- T cells which comprises:

a) obtaining peripheral blood T cells from a subject;

10 b) stimulating by multiple priming a T cell line from the T cells obtained in step (a) with autologous antigen presenting cells (APCs) pulsed with an allopeptide, said allopeptide comprising an amino acid sequence comprising both MHC class I and MHC class II amino acid sequences wherein the amino acid sequences are binding sequences and are recognized by the primed T cell line;

c) isolating primed CD8+ T cells and CD4+ T helper cells from the T cell line stimulated in step (b);

20 d) isolating primed CD8+CD28- T cells from the isolated primed CD8+ T cells of step (c);

25 e) detecting suppression by the primed CD8+CD28- T cells isolated in step (d) of interaction between the CD4+ T helper cells isolated in step (c) and autologous antigen presenting cells (APCs) expressing the same MHC class I and MHC class II binding motifs as expressed by the APCs used to stimulate the T cell line of step (b), thereby identifying allopeptide antigen specific human suppressor  
30 CD8+CD28- T cells; and

f) expanding the allopeptide antigen specific human suppressor CD8+CD28- T cells identified in step (e), thereby generating the allopeptide antigen specific human suppressor CD8+CD28- T cells.

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10. The method of claim 9 wherein the allopeptide is selected from the group consisting of a peptide antigen, a whole protein antigen, tat-DR4 peptide or a peptide comprising an amino acid sequence of a hypervariable region of HLA-DR B1.

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11. Antigen specific human suppressor CD8+CD28- T cells produced by the method of claim 9.

12. A method of determining whether a level of immunosuppresant therapy given to a subject undergoing the level immunosuppression therapy requires a reduction which comprises:

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a) obtaining a blood sample from the subject; and

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b) determining the presence of T suppressor cells present in the sample,

the presence of T suppressor cells indicating that the subject requires the reduction of immunosuppresant therapy.

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13. The method of claim 12 wherein the T suppressor cells are suppressor CD8+CD28- T cells.

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14. A method of reducing the risk of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises:

- 5 a) obtaining a blood sample from the subject;
- b) removing T suppressor cells from the blood sample;
- c) expanding the T suppressor cells of step (b); and
- 10 d) reintroducing the expanded T suppressor cells of step (b) into the subject.

15. The method of claim 14 wherein the T suppressor cells are suppressor CD8+CD28- T cells.

16. A method of reducing the level of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises administering to the subject the T suppressor cells produced by the method of claim 1, thereby preventing rejection of the tissue or organ transplant in the subject.

17. A method of reducing the level of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises administering to the subject the T suppressor cells produced by the method of claim 9, thereby preventing rejection of the tissue or organ transplant in the subject.

18. A method of preventing rejection of an allograft in a subject which comprises:

- a) obtaining a blood sample from the subject;

b) removing T suppressor cells from the blood sample;

c) expanding the T suppressor cells of step (b); and

d) reintroducing the expanded T suppressor cells of step  
(b) into the subject,

thereby preventing the rejection of the allograft in the  
subject.

18. A method of preventing rejection of an allograft in  
a subject which comprises administering the T  
suppressor cells produced by the method of claim 1  
to the subject, thereby preventing rejection of the  
allograft in the subject.

19. A method of preventing rejection of an allograft in  
a subject which comprises administering the T  
suppressor cells produced by the method of claim 9  
to the subject, thereby preventing rejection of the  
allograft in the subject.

20. A method of preventing rejection of a xenograft in  
a subject which comprises:

a) obtaining a blood sample from the subject;

b) removing T suppressor cells from the blood sample;

c) expanding the T suppressor cells of step (b); and

d) reintroducing the expanded T suppressor cells of step (b) into the subject,

thereby preventing the rejection of the xenograft in the subject.

21. The method of claim 20 wherein the T suppressor cells are suppressor CD8+CD28- T cells.

22. A method of preventing rejection of a xenograft in a subject which comprises administering the T suppressor cells produced by the method of claim 5 to the subject, thereby preventing rejection of the xenograft in the subject.

23. A method of preventing autoimmune disease in a subject which comprises:

a) obtaining a blood sample from the subject;

b) removing T suppressor cells from the blood sample;

c) expanding the T suppressor cells of step (b); and

d) reintroducing the expanded T suppressor cells of step (b) into the subject,

thereby preventing autoimmune disease in the subject.

24. The method of claim 23 wherein the T suppressor cells are suppressor CD8+CD28- T cells.



25. A method of preventing autoimmune disease in a subject which comprises administering the T suppressor cells produced by the method of claim 1 to the subject, thereby preventing autoimmune disease in the subject.

26. A method of preventing autoimmune disease in a subject which comprises administering the T suppressor cells produced by the method of claim 9 to the subject, thereby preventing autoimmune disease in the subject.

27. A vaccine comprising allospecific T suppressor cells stimulated by APCs expressing an MHC class I antigen and an MHC class II antigen which T suppressor cells suppress an interaction between CD4+ T helper cells and allogeneic antigen presenting cells (APCs) expressing the same MHC class I antigen and the same MHC class II antigen expressed by the APCs used to stimulate the allospecific T suppressor cells.

28. The vaccine of claim 27 wherein the APCs are allogeneic APCs said APCs expressing an MHC class I antigen recognized by the T suppressor cells and an MHC class II antigen recognized by allogeneic CD4+ T helper cells.

29. The vaccine of claim 27 wherein the APCs are APCs pulsed with an allopeptide, said allopeptide comprising an amino acid sequence having both MHC class I and MHC class II binding motifs wherein

both motifs are recognized by the stimulated T suppressor cells.

30. The vaccine of claim 27 wherein the T suppressor cells are suppressor CD8+CD28- T cells.

31. A vaccine comprising xenospecific T suppressor cells stimulated by APCs expressing a xenospecific MHC class I antigen and a xenogeneic MHC class II antigen which xenogeneic T suppressor cells suppress an interaction between CD4+ T helper cells and xenogeneic antigen presenting cells (APCs) expressing the same xenogeneic MHC class I antigen and xenogeneic MHC class II antigen expressed by the APCs used to stimulate the xenospecific T suppressor cells.

32. The vaccine of claim 31 wherein the T suppressor cells are suppressor CD8+CD28- T cells.

33. A method of inducing anergic T helper cells which comprises:

a) incubating antigen presenting cells (APC) with allospecific T suppressor cells (Ts);

b) overexpressing in the APC mRNA which encodes at least one monocyte inhibitory receptor (MIR), in a mixture of cells comprising the APCs from step (a), wherein overexpression of MIR transmits negative inhibitory signals to recruit an inhibitory signaling molecule, tyrosine phosphatase SHP-1 such that the APC are rendered tolerogenic; and

c) incubating the APCs from step (b) with T helper cells (Th) to induce Th anergy.

34. The method of claim 33, wherein the monocyte inhibitory receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

35. The method of claim 33, wherein the Ts are allospecific human suppressor CD8+CD28- T cells.

36. The method of claim 33, wherein the Ts are xenospecific human suppressor CD8+CD28- T cells.

37. The method of claim 33, wherein the Ts allopeptide are antigen specific human suppressor CD8+CD28- T cells.

38. A method of generating a tolerogenic antigen presenting cell (APC) which comprises:

- a) contacting the APC with Ts; and
- b) overexpressing mRNA which encodes an MIR in the APC, thereby generating a tolerogenic antigen presenting cell (APC).

39. The method of claim 38, wherein the monocyte inhibitory receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

40. The method of claim 38, wherein the Ts are antigen specific allospecific human suppressor CD8+CD28- T cells.

41. The method of claim 38, wherein the Ts are xenospecific human suppressor CD8+CD28- T cells.

5 42. The method of claim 38, wherein the Ts are allopeptide antigen specific human suppressor CD8+CD28- T cells.

10 43. A method of reducing the level of rejection of an allograft tissue or organ in a subject who is a transplant recipient of the allograft tissue or organ which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte inhibitory receptor (MIR), wherein the APC have been incubated with Ts prior to overexpression of MIR, thereby inducing Th anergy so as to prevent rejection of the tissue or organ allograft in the subject.

15 20 44. The method of claim 43, wherein the monocyte inhibitory receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

25 45. The method of claim 43, wherein the Ts are allospecific human suppressor CD8+CD28- T cells.

46. The method of claim 43, wherein the Ts are xenospecific human suppressor CD8+CD28- T cells.

30 47. The method of claim 43, wherein the Ts are allopeptide antigen specific human suppressor CD8+CD28- T cells.

48. A method of suppressing an autoimmune disease in a subject which comprises:

- 5 a) contacting antigen presenting cells (APC) of the subject with T suppressor cells (Ts) specific for the antigen which induces the autoimmune disease; and
- b) administering to the subject the APC of step(a), thereby inducing tolerance to the antigen so as to suppress the autoimmune disease in the subject.

10 49. The method of claim 48, wherein the monocyte inhibitory receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

15 50. The method of claim 48, wherein the Ts are allospecific human suppressor CD8+CD28- T cells.

20 51. The method of claim 48, wherein the Ts are xenospecific human suppressor CD8+CD28- T cells.

25 52. The method of claim 48, wherein the Ts are allopeptide antigen specific human suppressor CD8+CD28- T cells.

30 53. A method of suppressing an autoimmune disease in a subject which comprises:

- a) overexpressing monocyte inhibitory receptor (MIR) in antigen presenting cells (APC) of the subject, which APC present the antigen which

induces the autoimmune disease and are genetically engineered to overexpress MIR; and  
b) administering to the subject the APC of step(a), thereby inducing tolerance to the antigen so as to suppress the autoimmune disease in the subject.

54. The method of claim 53, wherein the monocyte inhibitory receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

55. A method of inducing tolerance to an allograft tissue or organ in a subject which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte inhibitory receptor (MIR), thereby inducing tolerance to the allograft in the subject.

56. A method of inducing tolerance to a xenograft tissue or organ in a subject which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte inhibitory receptor (MIR), thereby inducing tolerance to the xenograft in the subject.

57. An antigen presenting cell (APC) which overexpresses ILT3, wherein the APC comprises a retroviral vector comprising a nucleic acid sequence which encodes ILT3 and overexpresses ILT3.

58. The APC of claim 57, wherein the APC is an APC from a subject who is a tissue or organ transplant donor.

5 59. A method of inducing tolerance to a xenograft tissue or organ transplant in a subject which comprises:

- 10 a) introducing to an antigen presenting cell (APC) of a tissue or organ transplant donor a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and
- 15 b) administering the APC of step (a) to the subject, thereby inducing tolerance to the xenograft in the subject.

20 60. The method of claim 59, wherein the vector is a retroviral vector.

25 61. A method of inducing tolerance to an allograft tissue or organ in a subject which comprises:

- 30 a) introducing to an antigen presenting cell (APC) of a tissue or organ transplant donor a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and
- b) administering the APC of step (a) to the subject, thereby inducing tolerance to the allograft in the subject.

62. The method of claim 61, wherein the vector is a retroviral vector.

63. A method of treating an autoimmune disease in a subject which comprises:

a) introducing to an antigen presenting cell (APC) of a subject having the autoimmune disease a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and

b) administering the APC of step (a) to the subject, thereby treating the autoimmune disease in the subject.

64. The method of claim 63, wherein the vector is a retroviral vector.

65. The method of claim 63, wherein the autoimmune disease is selected from the group consisting of diabetes, rheumatoid arthritis, and multiple sclerosis.

66. A method of determining the appearance of T suppressor (Ts) cells which comprises detecting the level of expression of ILT3, ILT4, and ILT2 protein in APCs of a subject, wherein the subject is a xenograft tissue or organ transplant recipient which comprises:

a) obtaining a sample from the subject; and  
b) detecting in the sample of step (a) overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein in the APC of the subject, wherein detection of overexpression



of mRNA which encodes the ILT3, ILT4, and ILT2 protein indicates the appearance of T suppressor cells in the subject.

5 67. The method of claim 66, wherein the Ts are xenospecific human suppressor CD8+CD28- T cells.

10 68. A method of determining the appearance of T suppressor (Ts) cells which comprises detecting the level of expression of ILT3, ILT4, and ILT2 protein in APCs of a subject, wherein the subject is an allograft tissue or organ transplant recipient which comprises:

- 15 a) obtaining a sample from the subject; and  
b) detecting in the sample of step (a) overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein in the APC of the subject, wherein detection of overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein indicates the appearance of T suppressor cells in the subject.

20 69. The method of claim 68, wherein the Ts are allospecific human suppressor CD8+CD28- T cells.